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Factors Affecting Buoyancy in the Eggs of *Bairdiella icistia* (Pisces: Sciaenidae)*

S.C. May

Hawaii Institute of Marine Biology; Kaneohe, Hawaii, USA

Abstract

Several aspects of buoyancy were studied in the normally pelagic eggs of the sciaenid fish *Bairdiella icistia* (Jordan and Gilbert). Spawning fish acclimated to a low salinity (15°/oo) produced larger, more buoyant eggs which had a higher water content than eggs produced by fish living in ordinary sea water (33°/oo S). Eggs fertilized in lower salinities were larger and more buoyant than eggs fertilized in higher salinities. The salinity of the medium during the first 5 to 7 min after fertilization had a lasting effect on egg buoyancy, but subsequent transfer to a different salinity also influenced buoyancy. Although egg buoyancy in this species can be influenced by the ambient salinity both before and after spawning, the capacity for adjusting buoyancy is limited, and eggs spawned in salinities lower than 30°/oo would probably sink.

Introduction

Most marine fish eggs are pelagic (Blaxter, 1969), but in salinities below a certain threshold these eggs sink. The salinity threshold for buoyancy is important when the lower salinity tolerance of pelagic fish eggs is being considered, because sinking eggs will encounter different environmental conditions, which may or may not be conducive to embryonic survival. In shallow waters, sinking eggs may come to rest on the bottom where potential hazards abound, such as low oxygen tensions, a profuse bacterial flora, predation, and sedimentation.

A previous paper showed that eggs of the bairdiella *Bairdiella icistia* (Jordan and Gilbert), a marine fish, could develop successfully in salinities of 15°/oo and above (May, in press). Despite this physiological capability to develop in low salinities, it was felt that in some cases the actual lower salinity threshold for successful development might be determined by egg buoyancy, depending on water depth and local conditions. The experiments described below were carried out to determine the lower salinity at which bairdiella eggs remained buoyant and the extent to which their buoyancy could be adapted to the ambient salinity both before and after spawning.

Material and Methods

Bairdiella icistia is native to the Gulf of California, but an introduced population occurs in the Salton Sea, a large saline lake in Southern California with a salinity of approximately 38°/oo (Lasker *et al.*, 1972) and an ionic composition somewhat different from that of ordinary sea water (Carpelan, 1961). Adult bairdiella were obtained from the Salton Sea and maintained in sea water in the aquarium of the Southwest Fisheries Center in La Jolla, California. Fish which had been held in sea water for at least a year were induced to mature by photoperiod manipulation and induced to spawn by hormone injections, as described elsewhere (May, in press). One group of fish was gradually shifted from full-strength sea water (33°/oo S) to diluted sea water (approximately 15°/oo S), while another group was maintained in full-strength sea water. The fish were allowed to mature under these conditions for 4 to 5 months before spawning was induced by hormone injections. During the period of maturation, salinities varied only slightly, and the temperature remained at approximately 22°C.

Eggs were fertilized artificially and incubated by methods previously described (May, in press). Test salinities were prepared as described earlier (May, in press) and remained within 0.5°/oo of the desired value; the temperature during fertilization and incubation was maintained at 24°C (within ± 0.2C°) in all experiments. Eggs from two fish acclimated to 33°/oo S were fertilized and incubated in various salinities: 15, 25, 35, and 45°/oo for eggs from one fish; and 20, 30, and 40°/oo for eggs from the other. Eggs from two fish acclimated to 15°/oo S were fertilized and incubated in salinities of 15, 20, 30, and 40°/oo. In a later experiment, eggs from a fish acclimated to 33°/oo S were fertilized in 15, 30, and 40°/oo S, and 5 to 7 min after fertilization some eggs were transferred from 15 to 30°/oo S, from 30 to 40°/oo S,

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and from 40 to 30°/oo S; controls were kept in the salinity of fertilization. Salinities below 15°/oo were not utilized, since bairdiella eggs cannot be fertilized under these conditions (May, in press).

The buoyancy of eggs was determined at various developmental stages by transferring 10 eggs to each of a series of 25 ml vials containing salinities differing by 1°/oo, and observing the vertical distribution of eggs in the different salinities. The "salinity of neutral buoyancy" was defined as the lowest salinity in which 50% or more of the eggs remained above mid-depth. The vials used to determine buoyancy were immersed in a 24°C water bath. Egg diameters were measured with an ocular micrometer, and percentage water by loss in weight after drying in an oven at 60°C. The numerical designation of embryonic stages follows May (in press).

Results

The median diameters of unfertilized eggs from two fish living in 33°/oo S were 718 and 735 µm, and those of eggs from two fish in 15°/oo S were 735 and 753 µm (all medians based on measurements of 30 eggs). The difference between pooled egg-size measurements from the two acclimation salinities (60 measurements each) was barely significant ($P = 0.048$, Mann-Whitney U test). Fertilized eggs in normal sea water, measured during the gastrula stage, were approximately 40 µm larger in diameter than unfertilized eggs, due to the formation of the perivitelline space. The diameters of fertilized eggs tended to be somewhat smaller in the higher salinities (Table 1): this held for both the total egg diameter and the diameter of the yolk exclusive of the perivitelline space, suggesting some water loss from the yolk in the higher salinities and some gain in 15°/oo S. Both total and yolk diameters differed significantly among salinities ($P < 0.01$, Kruskal-Wallis test).

Eggs spawned by fish acclimated to 15°/oo S were more buoyant than those spawned by fish from 33°/oo S, and within each acclimation salinity, eggs fertilized and incubated in lower salinities were more buoyant than those in higher salinities (Fig. 1). Buoyancy measurements seem to reflect the fertilization salinity more than do measurements of diameter, perhaps because diameters were measured in these experiments only to the nearest 17.5 µm. The percentage of water was measured in unfertilized eggs stripped from 3 fish acclimated to 15°/oo S and from 4 fish acclimated to 33°/oo S: the 15°/oo S group produced eggs with a slightly higher water content than the 33°/oo S group (Table 2). The physiological salinity tolerance of the eggs was not influenced by parental salinity acclimation (May, in press).

When eggs were transferred from 15 to 30°/oo S within 5 to 7 min after fertilization, the median diameter of the eggs at gastrulation was significantly larger than that of eggs kept in 30°/oo S continuously (Table 3). The effect of salinity at fertilization was not noticeable in the diameters of eggs transferred from 40 to 30°/oo S. However both the 15 to 30°/oo S transfer group and the 4 to 30°/oo S group showed the effect of their fertilization salinities when their buoyancy was compared to that of eggs kept in 30°/oo S. The 1 to 30°/oo S group was more buoyant, and the 40 t 30°/oo S group less buoyant, than the control 3 to 30°/oo S group (Fig. 2). Conversely, the eggs transferred from 15 to 30°/oo S were less buoyan than the eggs kept in 15°/oo S, and the eggs tra ferred from 40 to 30°/oo S were more buoyant th

Table 1. *Bairdiella icistia*. Median egg diameters at Stage IIIa (early gastrula) in various salinities (eggs from fish acclimated to 15°/oo S). Measurements were made of total egg diameter and of diameter of yolk mass without perivitelline space. 12 eggs measured at each salinity; median diameter of unfertilized eggs = 753 µm

Salinity (°/oo)	Measurement (mm)	
	Total diameter	Yolk diameter
15	805	770
20	770	753
30	788	735
40	761	709

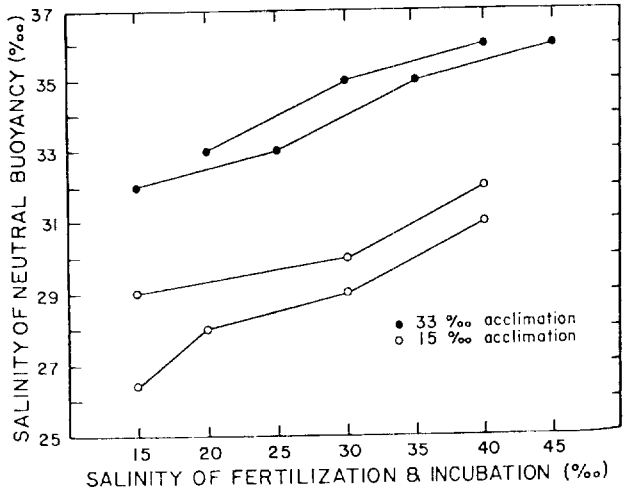

Fig. 1. *Bairdiella icistia*. Buoyancy of eggs fertilized and incubated in various salinities, and obtained from fish acclimated to 15 and to 33°/oo S. Lines connect values for eggs from individual fish (2 at each acclimation salinity). Buoyancy was measured between Stages IIe (blastula) and IIIa (early gastrula)

Table 2. *Bairdiella icistia*. Percentage water in unfertilized eggs obtained from fish acclimated to salinities of 15 and 33°/oo S. Each measurement is for eggs from a different fish

Acclimation salinity (°/oo)	Percentage water Measurements				Mean
15	90.4	91.6	91.3		91.1
33	89.7	90.2	90.0	88.9	89.7

Table 3. *Bairdiella icistia*. Nonparametric comparison of egg diameters by simultaneous test procedure (Sokal and Rohlf, 1969; p. 396). Eggs were either kept in constant salinity or transferred to different salinities shortly after fertilization. First value in treatment designation indicates salinity of fertilization; second, salinity of incubation. In each treatment, 30 eggs were measured at Stage IIIa (early gastrula). Medians not underscored by same line differ significantly at 5% level

Treatment (°/oo):	15-15	15-30	40-40	30-30	40-30
Median diameter (μm):	718	700	692	674	665

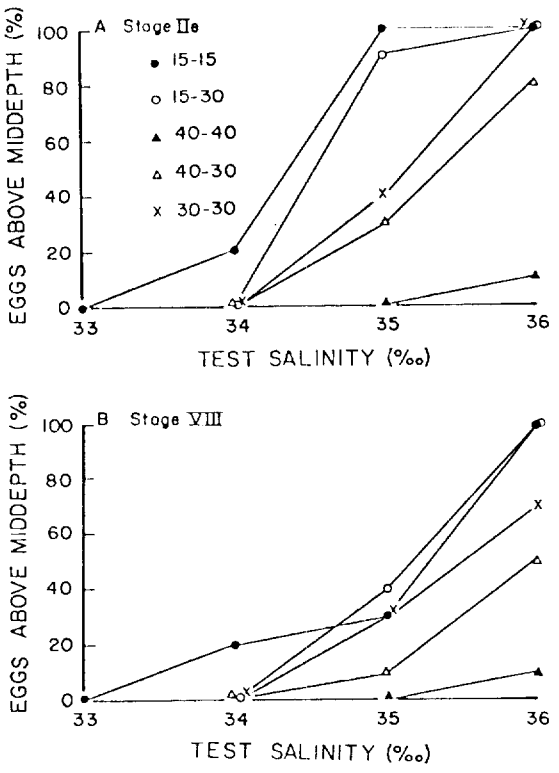

Fig. 2. *Bairdiella icistia*. Buoyancy of eggs transferred to new salinity between 5 and 7 min after fertilization. Some eggs were kept in salinity of fertilization and served as controls. Symbols: first value is salinity of fertilization; second, salinity of incubation. The vertical distribution of eggs in various salinities was noted at the blastula stage (Stage IIe) and (for the same eggs) shortly before hatching (Stage VIII), and is expressed as percentage of eggs remaining above middepth in 25 ml vials

those kept in 40°/oo S. These differences were apparent at Stage IIe (blastula) as well as at Stage VIII (shortly before hatching). The eggs became slightly less buoyant as hatching approached (see Fig. 2), probably due to the gradual utilisation of the low-density yolk (Franz, 1910). When compared with eggs obtained in earlier experiments, those used in the transfer study were slightly smaller, contained less water at spawning, and were less buoyant. This may indicate that the fish supplying the eggs had passed the peak of ripeness before spawning was induced.

Discussion

The "salinity of neutral buoyancy" for eggs spawned by *Bairdiella icistia* living in 33°/oo S can be estimated by interpolation to be approximately 34 to 35°/oo S for the three batches of eggs studied, i.e., it is above the salinity in which the fish were living. However, since the "salinity of neutral buoyancy" is here defined as the lowest salinity in which 50% or more of the eggs remain above mid-depth in 25 ml vials, some eggs will be suspended in the water column in 33°/oo S, even though this is below the "salinity of neutral buoyancy". Agitation in the water column might keep even more eggs suspended. Furthermore, the eggs used in these experiments were obtained from artificially-induced spawning of fish held for long periods of time in captivity and may, therefore, differ in unknown ways from naturally-spawned eggs.

In spite of the foregoing remarks, one may conclude that bairdiella eggs spawned in normal sea water have little reserve buoyancy to spare. Results presented in this paper show that acclimation of spawning fish to low salinity can increase the buoyancy of their eggs, but the increase is relatively small compared to the magnitude of the decrease in acclimation salinity (eggs

spawned by fish living in 15°/oo, and fertilized in that salinity, had a neutral buoyancy of 27 to 29°/oo S). From these data it seems unlikely that bairdiella living in a salinity below about 30°/oo could produce buoyant eggs, even considering the adjustments made possible by parental acclimation. This may therefore be the approximate lower salinity limit for successful reproduction in this species in shallow areas where the bottom is not conducive to embryonic survival.

Lönning and Solemdal (1972) showed that variations in chorion thickness (apparently determined genetically) allowed flatfish eggs to float in relatively low salinities in the Baltic Sea. Different opinions, however, have been expressed concerning nongenetic factors which may influence buoyancy in pelagic fish eggs. Solemdal (1967, 1971) stressed that the buoyancy of eggs was determined prior to spawning by the osmotic concentration of the body fluids. Hohendorf (1968) argued that buoyancy was determined after spawning, by the influx of ambient sea water into the perivitelline space. The present results with bairdiella eggs show that both of these non-genetic mechanisms can influence egg buoyancy.

The manner in which parental salinity acclimation affects egg buoyancy has not been fully elucidated. Solemdal (1967) measured a lower osmotic pressure in blood serum from flounders acclimated to a low salinity (5°/oo); he therefore attributed the change in egg buoyancy which was brought about by parental salinity acclimation, to a difference in the osmotic pressure of the blood, and hence the ovarian fluid, of spawning females. A gradual decrease in the blood osmotic pressure could explain the gradual increase in buoyancy noted in eggs produced by flounder after transfer from 34.5°/oo to 5 or 6.5°/oo S (Solemdal, 1967, 1971). In the present investigation, however, no difference in the freezing point depression of the blood was detected between bairdiella living in 15°/oo S and those living in 33°/oo S (May, in press), although the former produced eggs with greater buoyancy than the latter. Hence the steady-state osmotic pressure of the blood need not be altered in order for parental salinity acclimation to influence egg buoyancy. It has been known since the time of Fulton (1898) and Milroy (1898) that fish ova absorb a large amount of water and increase greatly in volume shortly before ovulation. Haydock (1971) showed that bairdiella gained as much as 13% of their initial body weight within 30 h after being injected with gonadotropic hormones. During this time the ovarian eggs increase in diameter from about 500 to 725 µm, which represents a threefold increase in volume. The fish must absorb a large quantity of water from the external environment during this period of rapid ovarian hydration, probably by drinking (Hirose *et al.*, 1974), and the osmotic pressure of the blood probably experiences a transient drop at this time, although no measurements of this phenomenon have been reported. The lower the salinity of the external medium, the easier (in an osmotic sense) it would be to absorb water from it. One might, therefore, expect a fish to acquire more water and to pass more water on to the ovarian eggs if the process of gonadal hydration were to take place when the fish is living in a relatively low salinity. This effect probably explains the higher percentage of water found in bairdiella eggs produced by fish living in 15°/oo S, even though the blood of these fish (not sampled during hydration) had the same freezing point depression as that of fish living in normal sea water. Tan's (1960) observations of a greater water content and lower concentration of ions in eggs from *Cottus bubalis* living in low-salinity water, may have a similar basis.

Hohendorf (1968) found a slight decrease in the freezing point depression of the blood of several fish species from lower salinities in the Baltic Sea, but did not find as great a decrease in the freezing point depression of their ovarian fluid as did Strodtmann (1918). The differences between these two studies could be due to the fish specimens being at different phases of the maturation cycle. From considerations brought out in the preceding paragraph, it is clear that fully ripe fish, if caught during the process of gonadal hydration, would likely have a lower blood osmotic pressure than fish before or after the postulated transient absorption of water into the blood, and the ovarian fluid would probably have a lower osmotic pressure after hydration than before. Strodtmann (1918; p. 43) in fact found decreasing freezing-point depressions as fish became more ripe, and Clemens and Grant (1964) found more ovarian water in ovulating than in nonovulating *Carassius auratus*.

Shelbourne (1956) emphasized that gonadal hydration satisfied the buoyancy and osmotic requirements of pelagic marine embryos. Hydration of the gonads prior to spawning appears, however, to be a more fundamental physiological event in fishes, occurring in species with demersal eggs as well as in those with pelagic eggs, and in freshwater as well as marine species. Male fish also show a gonadal hydration response at spawning and after injection with gonadotropic hormones (Clemens and Grant, 1964). Fulton (1898) hypothesized that the primary function of ovarian hydration was to dissolve the germinal vesicle, and that only secondarily had the process been utilized to render eggs pelagic.

The present experiments also show that the salinity at the time of fertilization affects egg buoyancy. This effect is probably related, as Hohendorf (1968) suggests, to the influx of ambient water into the perivitelline space during activation of the egg (Yamamoto, 1961). Within 4 min after fertilization, a small space is visible at the periphery of the blastodisc in bairdiella eggs (see Fig. 5a in May, in press). Since the chorion is freely permeable to both water and salts (Holliday, 1969), the fluid filling this space is the same as the ambient medium. Movement of water along the osmotic gradient between yolk and perivitelline fluid probably accounts for the observed differences in yolk diameters in different

salinities. Although the salinity of the medium at the time of fertilization affects the buoyancy of the bairdiella egg until hatching, transferring the fertilized egg to a different salinity will alter its buoyancy to a limited extent. It is possible that, after fertilization, the vitelline membrane of bairdiella eggs decreases rapidly in permeability but remains at least slightly permeable throughout development, as Zotin (1965) found in salmon eggs.

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Dr. R.C. May
Hawaii Institute of Marine Biology
P.O. Box 1346
Kaneohe, Hawaii 96744
USA

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